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Characterization and differentiation of five "*Vinhos Verdes*" grape varieties on the basis of monoterpenic compounds

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Abstract

The aromatic profiles of three white grape varieties (*Alvarinho*, *Loureiro*, and *Avesso*) and two red varieties (*Amaral* and *Vinhão*) from the *Vinhos Verdes* region have been established with respect to the monoterpenic compounds, present either in free and in glycosidically bound fractions. Seventeen compounds have been identified and quantified in the free form and 21 in the glycosidically bound form. *Loureiro* variety is characterized by important levels of linalool in the free fraction, above the odour perception threshold; in contrast, *Alvarinho* and *Avesso* varieties do not contain compounds above the perception threshold. For *Alvarinho*, geraniol prevails, followed by linalool, while *Avesso* only has, in much low concentration, geraniol, nerol and citronellol; red varieties do not contain terpenic compounds. *Loureiro* and *Alvarinho* are still the richer varieties with regard to the glycosylated fraction; linalool and 3,7-dimethylocta-1,5-dien-3,7-diol have equivalent concentrations, and linalool is around the odour perception threshold; *Avesso* does not contain linalool. The isomers (*Z*) and (*E*) of 8-hydroxylinalool seem to differentiate white varieties; they are similar in *Loureiro* but the (*Z*) isomer prevails in *Avesso* and especially in *Alvarinho*; *Avesso* has a more balanced distribution. *Amaral* only contains α-terpineol, nevertheless of similar concentrator found in *Loureiro*; *Vinhão* has a more balanced distribution of isomers but the concentrations are very low.

The results show that profiles of the terpenic compounds vary to a significant degree for the grape varieties studied and as is already known empirically, the white varieties are richer than red varieties, especially *Loureiro*.

Keywords: Terpenols; *Alvarinho*; *Loureiro*; *Avesso*; *Amaral*; *Vinhão*; Wine

1. Introduction

Wines with Appellation of Origin "*Vinhos Verdes*" are produced in a very wide area in Portugal, the broadest of Europe, composed by nine sub-regions (Amarante, Ave, Baião, Basto, Cávado, Lima, Monção, Paiva and Sousa). White wines, characterized by the freshness and the floral and fruity flavours, have already acquired international recognition; red wines are known mainly by their colour and marked astringency and are basically consumed with regional food. There are seven recommended white grape varieties (*Alvarinho*, *Arinto*, *Avesso*, *Azal*, *Batoca*, *Loureiro* and *Trajadura*) and eight red grape varieties (*Amaral*, *Borraçal*, *Brancelho*, *Espadeiro*, *Padeiro de Basto*, *Pedral*, *Rabo de Ovelha* and *Vinhão*) used to produce these wines.

It is well known that monoterpenic compounds are important to discriminate between grape varieties. In fact, several authors [1,2] could differentiate *Riesling* wines from different regions, or select between *Riesling* and *Gewürztraminer* clones, based on the monoterpenic composition of grapes. Strauss et al. [3] have also classified *Vitis vinifera* cultivars in Muscat varieties, in non-Muscat aromatic varieties and in neutral varieties, based on global concentration of monoterpenic compounds (free plus glycosidically bound); on the other hand, Rapp [4] has classified German varieties in three groups—"*Riesling* type", "*Muscat*-type" and "*Sylvaner*-type"— by the quantification of 12 monoterpenic compounds.

Terpenic compounds can be present either in a free volatile form or in a glicosidically bound form [5–7]. Glycosides

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may be constituted by glucose or by glucose and rhamnose, arabinose or apiose, linked to the aromatic aglycon, and can be hydrolysed by specific enzymes [8–10]. To date, about 40 terpenic compounds have been identified in berry grapes, belonging to various chemical families such as hydrocarbons, alcohols, esters, aldehydes and acids [11,12]. Monoterpenols, for example linalool, HO-trienol, α-terpineol, citronellol, nerol and geraniol, as well as some oxides such as roseoxide and neroloxide, are among the most aromatic, with fruity and floral flavours and low perception thresholds [12,13]. Other monoterpenic alcohols, with two or three alcohol functions, and other oxides, can not contribute significantly to the overall flavour of grapes and wines but they can act as markers for each variety. They may play an important role, similar to glycosides, in the flavour of the future wine, as they can act as precursors of volatile compounds [14,15].

The full knowledge of *Vinhos Verdes* grape varieties, namely aromatic characterization, becomes important as it may serve to discriminate these autochthonous varieties and to better explore their own potential to produce high quality wines. In this context, some results for *Loureiro* and *Alvarinho* varieties have already been published [16]. The aim of this work is to continue the characterisation of these two cultivars and extend the study to other recommended grape varieties, namely *Amaral*, *Aveso* and *Vinhão*, to determine the monoterpenic composition of free and glycosidically bound fractions of the grapes.

2. Experimental

2.1. Grape samples

About 2 kg of each variety—*Alvarinho*, *Amaral*, *Avesso*, *Loureiro* and *Vinhão*—were manually picked at random, at technological maturity on the dates, places and rootstocks referred to in Table 1. Each variety was only studied for 1 year, one rootstock and one sub-region, in order to obtain the most representative samples in the author's opinion. All the samples studied were from 2001 vintage, except for *Alvarinho*, that was from 1998 vintage. The rootstock was always the 1103 P, being the 110 R for *Avesso* cultivar.

The berries were picked manually from grape clusters, selected, frozen in liquid nitrogen and stored at −20 °C. For each sample the average berry weight, total acidity, pH and sugar content were determined using standard procedures.

2.2. Solvents

All solvents were analytical grade and further purified. Ethyl acetate (Merck, ref. 1.09623) and diethyl ether (Merck, ref. 1.00921) were distilled, the last one over iron(II) sulphate (Merck, ref. 1.03965). Dichloromethane (Merck, ref. 1.06050) was washed with de-ionised water, and then distilled. Pentane (Carlo Erba, ref. 468151) was washed with H_2SO_4, $KMnO_4$ and de-ionised water, and next was distilled over potassium hydroxide (Merck, ref. 1.05033). Azeotrope pentane–dichloromethane (2:1, v/v) was distilled after combination and redistilled whenever necessary.

2.3. Extraction of free and bound fractions

About 550 g of frozen berries were thawed at 4 °C overnight, then crushed in a blender (turbo blender, Moulinex, position 4, 7 s), centrifuged (RCF = 9660, 25 min, 4 °C) and filtered. The yield of juice production was determined (Table 2). To 100 ml of juice, 14.5 μg of 4-nonanol (Merck, ref. 818773) were added and passed through an Amberlite XAD-2 resin (20–60 mesh, Supelco, ref. 1-0357) column according to Günata et al.

Table 1
Date and place of harvest, rootstock and sub-region for each variety studied

	Date of harvest	Rootstock	Training system	Sub-region
Alvarinho	21 September 1998	1103 P	Single cordon	Monção
Amaral	28 September 2001	1103 P	Single cordon	Amarante
Avesso	4 October 2001	110 R	Single cordon	Baião
Loureiro	11 October 2001	1103 P	Single cordon	Lima
Vinhão	16 October 2001	1103 P	Single cordon	Lima

Table 2
Global characterization of grapes and juices

	Alvarinho	*Amaral*	*Avesso*	*Loureiro*	*Vinhão*
pH	3.03	2.69	2.80	3.06	3.02
Total acidity[a] ($mg\,l^{-1}$)	8.0	13.54	9.65	9.92	10.60
Sugar content ($g\,l^{-1}$)	211	168	211	164	173
Mean berry weight[b] (g)	1.13 (1690)	1.48 (500)	1.80 (500)	1.55 (500)	1.92 (500)
Juice efficiency ($ml\,kg^{-1}$)	656	660	680	560	682

[a] Expressed as tartaric acid.
[b] Values in brackets refer to the number of berries weighted.

[17], and modified by Oliveira [18]. Free and bound fractions were eluted successively with 50 ml of azeotrope pentane–dichloromethane and 50 ml of ethyl acetate. The pentane–dichloromethane eluate was dried over anhydrous sodium sulphate and concentrated to 200 μl by solvent evaporation at 34 °C through a Vigreux and then a Dufton column, prior to analysis. The ethyl acetate eluate was concentrated to dryness in vacuum (40 °C) and dissolved in 0.2 ml of 100 mmol l^{-1} citrate–phosphate buffer (pH = 5.0). Residual free compounds were extracted five times with pentane–dichloromethane and discarded. Fourteen milligrams of enzyme AR2000 (Gist-Brocades) were added to the glycosidic extract and the mixture was incubated at 40 °C, for 12 h. Released aglycons were extracted with pentane–dichloromethane. 11.6 μg of 4-nonanol, as standard, was added to the organic phase and concentrated to 200 μl through a Dufton column. Analyses were made in triplicate.

2.4. Chromatographic analysis

Gas chromatographic analysis of free and released volatile compounds were performed using a gas chromatograph–mass spectrometer constituted by a Varian 3400 chromatograph and a Varian Saturn II ion-trap mass spectrometer. Each 1 μl injection was made in a capillary column, coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 film thickness, Chrompack). The temperature of the injector (SPI, septum-equipped programmable injector) was programmed from 20 °C to 250 °C, at 180 °C min^{-1}. The temperature of the oven was held at 60 °C, for 5 min, then programmed to rise to 250 °C, at 3 °C min^{-1}, then held 20 min at 250 °C and finally programmed to rise to 255 °C at 1 °C min^{-1}. The carrier gas was helium N60 (Air Liquide), at 103 kPa. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 *m/z* to 360 *m/z*, and an acquisition time of 610 ms.

2.5. Identification and quantification of monoterpenic compounds

Identification was preformed using the software Saturn version 5.2 (Varian), by comparing mass spectra and retention times with those of pure standard compounds. All the compounds were quantified as 4-nonanol equivalents.

3. Results and discussion

3.1. General analysis

pH, total acidity, sugar content, mean berry weight and juice yield for the five varieties studied are summarised in Table 2. The sugar content is higher for the *Alvarinho* and *Avesso* varieties, and the mean berry weight and juice efficiency are higher for the *Avesso* and *Vinhão* cultivars. Total acidity is quite high for all the samples. These results are in agreement with some published data [19–21].

3.2. Free monoterpenic compounds

GC–MS analysis allowed the identification of 17 monoterpenic compounds in the free fraction. Table 3 shows the mean level and the limits, with 95% confidence, obtained for each compound in the five varieties studied. The monoterpenic profile for each variety is shown in Fig. 1. For this

Table 3
Mean levels (*C*) and limits, with 95% confidence, for the monoterpenic compounds found in the free fraction of the five varieties studied

		Alvarinho		*Amaral*		*Avesso*		*Loureiro*		*Vinhão*	
		C (μg l^{-1})	±	*C* (μg l^{-1})	±	*C* (μg l^{-1})	±	*C* (μg l^{-1})	±	*C* (μg l^{-1})	±
1	*trans*-Furan linalool oxide	0.2	0.1	–		–		4.6	1.6	–	
2	*cis*-Furan linalool oxide	–		–		–		3.2	2.0	–	
3	Linalool	10.7	0.4	–		–		239.0	75.2	–	
4	4-Terpineol	–		–		–		–		1.2	0.6
5	HO-Trienol	0.3	0.3	–		–		–		–	
6	α-Terpineol	0.4	0.2	–		–		3.5	1.0	–	
7	*trans*-Pyran linalool oxide	6.0	1.4	–		–		44.8	11.4	0.3	0.2
8	*cis*-Pyran linalool oxide	0.4	0.2	–		–		8.4	1.9	–	
9	Citronellol	1.3	0.8	–		1.3	0.4	–		–	
10	Nerol	1.6	0.3	–		1.5	0.6	0.2	0.0	0.3	0.2
11	Geraniol	25.1	6.8	7.8	0.6	13.0	0.2	3.6	3.1	–	
12	*exo*-2-Hydroxy-1,8-cineole	–		–		–		2.5	1.6	–	
13	3,7-Dimethylocta-1,5-dien-3,7-diol	2.4	1.8	–		–		5.2	4.5	–	
14	3,7-Dimethylocta-1,7-dien-3,6-diol	1.5	0.9	–		–		3.3	1.5	–	
15	8-Hydroxy-6,7-dihydrolinalool	–		–		–		–		1.0	0.2
16	(*Z*)-8-Hydroxy-linalool	0.6	0.5	–		–		–		–	
17	Geranic acid	4.4	1.1	–		–		–		–	
	Total	54.9		7.8		15.8		318.3		2.8	

(–) not detected.

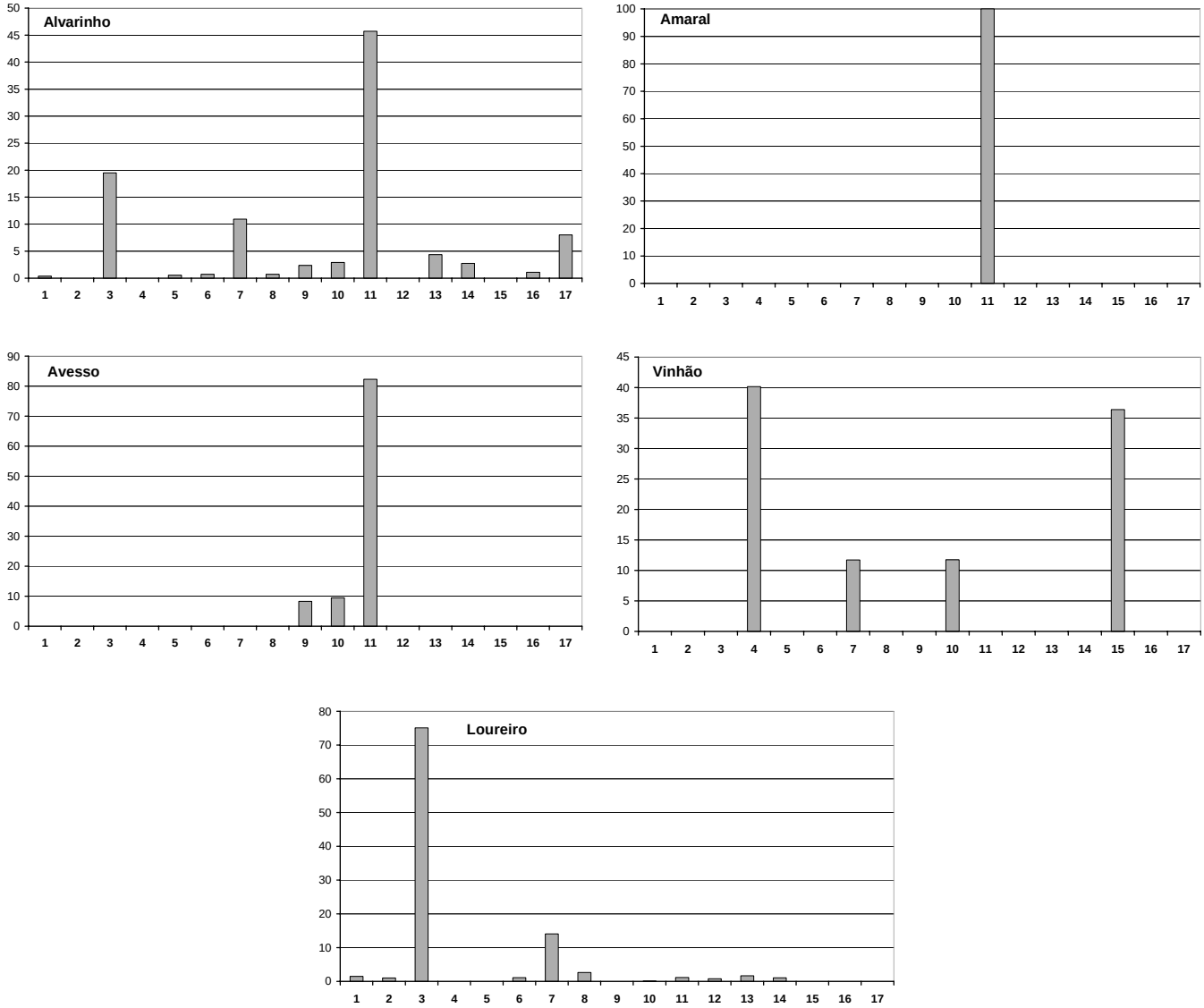

Fig. 1. Monoterpenic profile of the free fraction for *Alvarinho*, *Amaral*, *Avesso*, *Loureiro* and *Vinhão* varieties (each bar corresponds to the percentage contribution of the compound to the total concentration).

purpose, the percentage contribution of each compound to the total concentration of monoterpenic compounds was considered.

With regard to the overall concentration of monoterpenic compounds, the *Loureiro* variety is the richest one, followed by *Alvarinho*, *Avesso*, *Amaral* and *Vinhão*.

For the *Loureiro* variety, linalool is present above its aromatic threshold level (50–100 $\mu g\,l^{-1}$) [12,22,23] and represents about 75% of the total monoterpenic concentration and about 97% of total monoterpenols (linalool + 4-terpineol + HO-trienol + α-terpineol + citronellol + nerol + geraniol); this has already been mentioned about other *Loureiro* samples, harvested in different years and obtained from different rootstocks, which permits classification of this cultivar among the aromatic varieties [16,18]. The linalool level seems to be higher than in aromatic varieties like *Riesling* and *Gewürztraminer*, and neutral varieties like *Chardonnay* and *Cabernet Sauvignon* [17,24]. The other compounds were not significant from the aromatic point of view.

As we can see in Fig. 1, the monoterpenic profiles are quite different for the five varieties. *Loureiro* shows its typical profile with a large predominance of linalool (75.1%), followed by *trans*-pyran linalool oxide (14.1%); the third most abundant compound is *cis*-pyran linalool oxide (2.6%) but it can vary according to vintage, rootstock and "*terroir*" [18]. *Alvarinho* has a more balanced distribution, geraniol being the most abundant monoterpene (45.7%), followed by linalool (19.5%) and then by *trans*-pyran linalool oxide (10.9%); geraniol comprises 64% of the total monoterpenols content but the level found (25.1 $\mu g\,l^{-1}$) is below its perception threshold, which is about 100 $\mu g\,l^{-1}$ [12,22]. Nevertheless, depending on the sub-region, vintage and "*terroir*", the second and third compounds may vary among linalool,

trans-pyran linalool oxide and geranic acid, the percentage difference between them always being small [18]. *Avesso* variety is very poor with respect to monoterpenic compounds; geraniol, has a weak concentration (13.0 $\mu g\,l^{-1}$), representing 82.3% of the total value. The red varieties, *Amaral* and *Vinhão*, also have very small contents of monoterpenic compounds: *Amaral* seems to be similar to *Avesso*, with geraniol representing almost 100% of the total; *Vinhão* just contains traces of some monoterpenes.

3.3. Glycosidically bound monoterpenic compounds

GC–MS analysis allowed 21 monoterpenic compounds to be determined in the glycosidically bound fraction. Table 4 shows the mean level and the 95% confidence limit obtained for each compound, for the five varieties studied. The monoterpenic profile for each variety is shown in Fig. 2. As for the free fraction, the contribution of each compound to the total concentration of monoterpenic compounds, was considered.

The levels of monoterpenic compounds for the *Loureiro* variety are higher than those published for the *Chardonnay* variety [24] and are similar to those referred for other non-Muscat aromatic varieties like *Müller-Thurgau* and *Gewürztraminer* [25,26]; however, the *Loureiro* variety contains more linalool and less nerol and geraniol when compared to *Gewürztraminer*.

Regarding monoterpenols, the distribution is similar to that observed for the free fraction, except for α-terpineol, which is more abundant in the glycosidically bound fraction. The linalool level is smaller than for the free fraction but, even so, above the perception threshold. Here, the most abundant oxide is *trans*-furan linalool oxide. 3,7-Dimethylocta-1,5-dien-3,7-diol is the diol with the highest concentration. The isomers (*E*) and (*Z*) of 8-hydroxy-linalool are present in similar amounts. The monoterpenic profile represented in Fig. 2 shows that linalool continues to be, as for the free fraction, the compound that contributes the highest percentage (32.6%), followed by 3,7-dimethylocta-1,5-dien-3,7-diol (23.8%). However, in some other cases (unpublished data) it can be the opposite.

Alvarinho variety had a monoterpenic profile quite similar to *Loureiro*'s, with analogous levels of linalool and 3,7-dimethylocta-1,5-dien-3,7-diol (ca. 15%); here, (*Z*)-8-hydroxy-linalool is the highest contributor to the overall concentration of monoterpenic compounds (39.3%). The content on this compound, 183 $\mu g\,l^{-1}$, is comparable to that reported for *Muscat of Frontignan* juices, and higher than that found in *Riesling*, *Gewürztraminer*, *Müller-Thurgau* and *Muscat of Alexandria* [7,26,27]. The most important monoterpenol is linalool, contrary to that observed in the free fraction, with a concentration near the perception threshold. The overall concentration of monoterpenic glycosides found in *Alvarinho* variety (466 $\mu g\,l^{-1}$) is larger than that reported for some non-Muscat *Vitis vinifera* varieties such as *Chardonnay*, *Gewürztraminer* and *Müller-Thurgau* [24–26].

Avesso variety had no significant levels of monoterpenic compounds in the bound fraction. As for the free

Table 4
Mean levels (*C*) and limits, with 95% confidence, for the monoterpenic aglycons found in the glycosidically bound fraction of the five varieties studied

		Alvarinho		*Amaral*		*Avesso*		*Loureiro*		*Vinhão*	
		C ($\mu g\,l^{-1}$)	±	*C* ($\mu g\,l^{-1}$)	±	*C* ($\mu g\,l^{-1}$)	±	*C* ($\mu g\,l^{-1}$)	±	*C* ($\mu g\,l^{-1}$)	±
1	*trans*-Furan linalool oxide	21.5	8.6	0.4	0.2	0.8	1.0	39.1	14.5	0.5	0.1
2	*cis*-Furan linalool oxide	7.2	2.4	0.8	0.4	3.2	1.5	4.0	2.7	2.2	0.0
3	Linalool	73.8	19.4	0.8	0.3	0.2	0.2	151.4	47.6	1.0	0.4
4	4-Terpineol	–		0.4	0.1	–		0.4	0.3	0.3	0.1
5	HO-Trienol	3.2	1.3	–		–		1.5	0.4	–	
6	α-Terpineol	2.7	0.7	19.0	4.6	4.1	1.3	20.5	4.6	0.2	0.1
7	*trans*-Pyran linalool oxide	14.8	4.5	0.3	0.1	1.2	0.4	11.6	5.0	0.5	0.4
8	*cis*-Pyran linalool oxide	3.5	1.1	0.6	0.3	1.1	0.2	1.9	1.0	1.2	0.7
9	Citronellol	0.3	0.1	0.2	0.1	1.4	0.6	0.2	0.2	–	
10	Myrtenol	0.2	0.0	–		1.2	0.6	0.9	0.6	–	
11	Nerol	2.5	0.5	0.9	0.1	2.6	2.1	2.3	0.6	0.3	0.3
12	Geraniol	16.8	2.6	6.9	2.1	13.7	6.0	3.8	1.2	1.9	1.4
13	*exo*-2-Hydroxy-1,8-cineole	0.4	0.1	1.3	0.9	3.1	3.0	3.5	2.3	0.2	0.0
14	3,7-Dimethylocta-1,5-dien-3,7-diol	72.5	7.0	0.5	0.3	1.2	1.1	110.5	109.6	0.3	0.4
15	Linalool hydrate	1.0	0.2	–		–		5.0	4.7	–	
16	3,7-Dimethylocta-1,7-dien-3,6-diol	9.4	1.0	–		–		15.9	8.3	–	
17	8-Hydroxy-6,7-dihydrolinalool + citronellol hydrate	7.8	1.0	0.7	0.3	4.3	3.0	7.0	2.4	1.5	0.7
18	(*E*)-8-Hydroxy-linalool	32.8	5.2	0.2	0.1	6.1	5.1	31.0	31.0	1.0	0.7
19	(*Z*)-8-Hydroxy-linalool	183.3	11.0	5.1	1.4	10.8	6.9	36.1	38.4	4.6	3.1
20	Geranic acid	10.9	0.9	1.1	0.3	5.6	1.4	1.6	2.0	0.5	0.4
21	*p*-1-Menthen-7,8-diol	1.4	0.1	1.9	0.5	8.1	8.7	15.6	23.5	0.1	0.0
	Total	466.0		41.1		68.7		463.8		16.3	

(–) not detected.

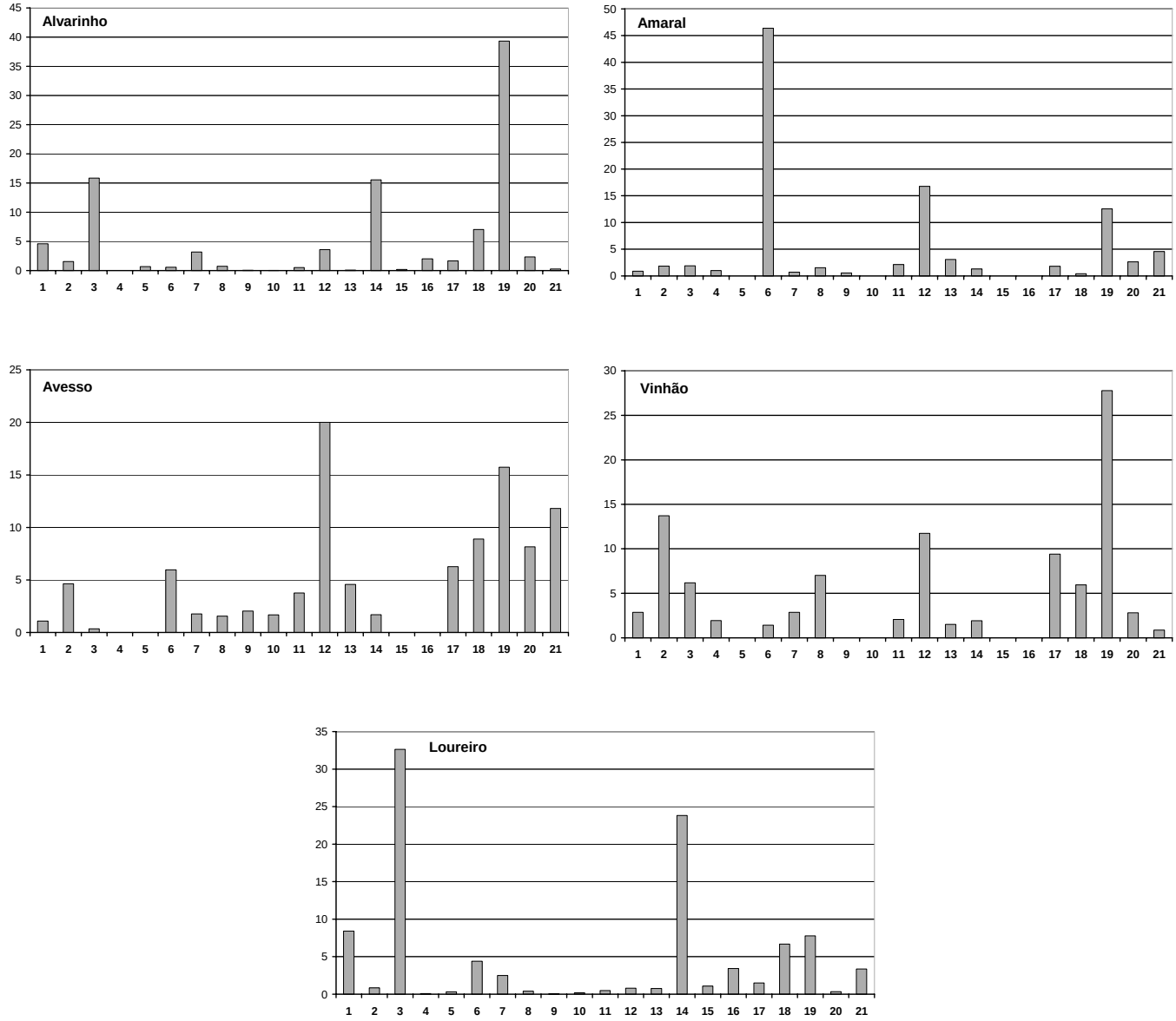

Fig. 2. Monoterpenic profile of the glycosidically bound fraction for *Alvarinho*, *Amaral*, *Avesso*, *Loureiro* and *Vinhão* varieties (each bar corresponds to the percentage contribution of the compound to the total concentration).

fraction, geraniol remains the most important compound but it is followed closely by (*Z*)-8-hydroxy-linalool and *p*-1-menthen-7,8-diol. However, for the same vintage, depending on the rootstock and "*terroir*", these positions can change. Geraniol accounts for 59% of total monoterpenols but its content, 13.7 $\mu g\,l^{-1}$, is much lower than the perception threshold (100–130 $\mu g\,l^{-1}$) [12,22]. It can also be seen that the monoterpenic profile is more balanced than those obtained for *Loureiro* and *Alvarinho* varieties.

Red grape varieties had much more discrete levels of monoterpenic compounds particularly *Vinhão*. For *Amaral*, α-terpineol is the most important compound, at 19 $\mu g\,l^{-1}$, and it accounts for 67.4% of total terpenols and 46.4% of the overall concentration. However, this level is not enough to reach the perception threshold 110–400 $\mu g\,l^{-1}$ [12,13]. The profile shows that α-terpineol is followed by geraniol and (*Z*)-8-hydroxy-linalool, these last two showing analogous percentages. *Vinhão* only contains traces of monoterpenic compounds, as observed for the free faction. Thus, it is difficult to establish the monoterpenic profile for this cultivar, as errors associated with the concentration determination can influence it significantly.

3.4. Validation of the profiles

It must be emphasised that the monoterpenic profiles established in this work are just for the 2001 vintage, with the exception of the *Alvarinho* variety. It can cause some doubts about the validity of these comparisons. However, results obtained for this cultivar in two vintages (1997 and 1998),

three rootstocks (161-49, 196-17 and 1103 P) and two different sub-regions (Lima and Monção) as well as for *Loureiro* in three vintages (1997, 1998 and 2001), three rootstocks (196-17, SO4 and 1103 P) and three sub-regions (Amarante, Lima and Cávado) [18] showed that the monoterpenic profile is quite similar for each situation, which permits the discussion and conclusions presented in this text. In the same way, the conclusions reported here for the other three varieties studied for the 2001 vintage are in agreement with other unpublished data obtained for two different rootstocks and two sub-regions: *Avesso* (Amarante and Baião; 110 R and 196-17), *Amaral* (Amarante and Lima; 161-49 and 1103 P) and *Vinhão* (Amarante and Lima; 161-49 and 1103 P).

4. Conclusions

The results show that it is possible to differentiate the recommended grape varieties for the *Vinhos Verdes* Region with regard to the quantification of monoterpenic compounds either in the free or in the glycosidically bound fraction.

For the five cultivars studied, this approach also provides proof that white varieties are much richer than red ones, either in free and in bound fractions. *Loureiro*, as is already known, is an aromatic variety because of the levels of linalool in the free fraction. *Alvarinho* variety, which is poorer than *Loureiro* with respect to the free fraction, presents interesting levels of terpenic compounds in the bound fraction, as well as *Loureiro*. This fact may become important in winemaking, since these compounds, particularly linalool, can be liberated from a glycoside moiety by specific enzymes and so contribute to the final wine flavour; other compounds such as oxides and diols, at the concentrations found in this study, may be rearranged at acidic pH to produce aromatic compounds.

The ratio between (*Z*) and (*E*) isomers of 8-hydroxylinalool present in the glycosidically bound fraction seems to be important in differentiating *Alvarinho* and *Loureiro* varieties, with values near 6 for the first cultivar and about unity for the second one.

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